International Journal of Infectious Diseases 18 (2014) 27–31

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Seroepidemiological survey of tularemia among different groups in western Iran

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ARTICLE INFO

Article history:
Received 2 June 2013
Received in revised form 7 August 2013
Accepted 23 August 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Tularemia
Francisella tularensis
Seroprevalence
Iran
ELISA

SUMMARY

Background: The first human case of tularemia in Iran was reported in 1980 and there have been no subsequent reports of tularemia in the country. The aim of this study was to carry out a survey of tularemia among different groups in the province of Kurdistan in western Iran.
Methods: The following information was collected by means of an in-house questionnaire: participant demographic characteristics, exposure to risks, and use of appropriate personal protective equipment and disinfectant in their occupation. A blood sample was collected from each participant. Sera were tested using an ELISA kit (Virion\Serion) to detect specific IgG antibodies against *Francisella tularensis*.
Results: Of a total of 250 serum samples, 14.40% had anti-tularemia IgG antibodies. The highest seroprevalence was found in hunters (18%) and the lowest in health care workers (12%). Age had a significant positive association with tularemia seroprevalence ($p < 0.001$). The seroprevalence of tularemia in people exposed to foxes (hunting or eating the meat) (25%) was significantly higher than in others (8.65%) ($p = 0.01$).
Conclusions: According to the findings of this study, it is highly recommended that physicians and health care workers are informed about bacteria circulating in this area. By sensitizing the health system, it is expected that some cases of the clinical disease will be reported in the near future. Similar studies in other parts of the country and on domestic and wild animals will clarify the epidemiology of tularemia in Iran.

1. Introduction

Tularemia is a zoonotic disease caused by the Gram-negative and intracellular bacterium *Francisella tularensis*. Because of its high infectivity and low infection dose, *F. tularensis* has been classified as one of the most dangerous pathogens by the US Centers for Disease Control and Prevention (Category A, CDC).[1,2] Clinical signs of the disease are more relevant to the subspecies *tularensis* and *holarctica* of *F. tularensis*.[1] Subspecies *tularensis* (type A) is the predominant cause of tularemia infection in the USA, and is the cause of an average of 124 new cases of tularemia in the USA annually.[3] Type A is reported to have a terrestrial cycle; the main reservoirs are rabbits and ticks.[4] Subspecies *holarctica* (type B) is responsible for almost all tularemia infections in Europe and Asia. Type B is reported to have a mainly water-borne cycle with aquatic rodents as reservoirs. Type B is associated with water and animals living near water.[4,5]

F. tularensis infection has been noted in a staggering number of wildlife species, including lagomorphs, rodents, arthropods (mainly ticks), carnivores, ungulates, marsupials, birds, amphibians, fish, and invertebrates, and also livestock, but the main sources of infection for humans are rodents and rabbits and the arthropods.[4,6] Tularemia can be transmitted to humans by direct contact with infected animals or their tissues, ingesting

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1201-9712
http://dx.doi.org/10.1016/j.ijid.2013.08.013

undercooked infected meat, or via contaminated water, animal bites or scratches, arthropod bites, and inhalation of aerosol or contaminated dust.[7,8] Tularemia causes a wide variety of clinical symptoms, usually related to the route of entry of the pathogen, and can manifest in asymptomatic to severe forms.[5] The common clinical forms of the disease include ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal (systemic) tularemia.[9] Clinical symptoms and virulence of the disease in type A is more frequent than in type B, and in general, mortality associated with untreated tularemia is 10–40% for type A and 1% for type B.[1,10] Although early identification of the pathogen is important, isolation by culture, detection of antigens, and molecular approaches are not always successful or appropriate.[11] Antibodies against tularemia appear 1 to 2 weeks after infection and these antibodies are detectable for several years after infection (10 to 20 years).[12,13] Therefore, the detection of antibodies against *F. tularensis* by serological tests such as ELISA is suitable for epidemiological studies on tularemia.[14]

In a study in 1973, tularemia antibodies were detected for the first time in Iran, in domestic animals (cattle and sheep) in the northwest and in a porcupine in the southeast.[15] The first report of human tularemia (glandular tularemia) in Iran was in the city of Marivan in the southwest of Kurdistan Province (in the west of Iran) in 1980. The patient was a soldier working in deserts and the clinical symptoms were fatigue, myalgia, headache, anorexia, chills, and enlarged inguinal lymph nodes.[16]

Due to the fact that tularemia is an endemic disease in Turkey (Iran's northwest neighbor) and several clinical cases of tularemia are reported annually from that country,[17] and because of the recent detection of tularemia antibodies in the human population of the Republic of Azerbaijan (Iran's northern neighbor),[18] and taking into account the fact that there is no updated information with respect to tularemia in Iran, the aim of this study was to investigate tularemia IgG among different groups in Kurdistan Province in western Iran.

2. Materials and methods

2.1. Study area and sampling

This study was carried out during 2011–2012 among different populations in Kurdistan Province, western Iran. Approximately 700 000 people lived in the study area. The sample units were selected based on a convenience sampling method. The sampling of this survey was from the western regions of this province, with a focus on the counties of Sanandaj, Marivan, and Sarvabad (Figure 1). The different groups of people surveyed included hunters and their families, butchers and slaughterhouse workers, health care workers, and those referred to medical diagnostic laboratories. All individuals enrolled in this study were over 18 years of age and were selected at random among their groups. After consent to participate in the study was obtained, the following information was collected by means of an in-house questionnaire: participant demographic characteristics (such as occupation, age, gender, and area of residence), exposure to risks (keeping animals, hunting or eating the meat of wild animals, length of employment, exposure to ill or dying animals, splashing animal fluids on face/body, and cuts to the hands during work), and the use of appropriate personal protective equipment and disinfectant in their occupation. On completion of the questionnaire, an 8-ml blood sample was collected from each participant and immediately transferred to the laboratory for separation of the serum. Serum samples were kept below −20 °C and transferred to the Department of Epidemiology of the Pasteur Institute of Iran (Tehran, Iran).

The proposal of this study was approved by the Ethics Committee of the Pasteur Institute of Iran.

2.2. Serological tests

Collected sera were tested for the detection of anti-tularemia IgG antibodies using a commercial ELISA kit (Virion\Serion GmbH,

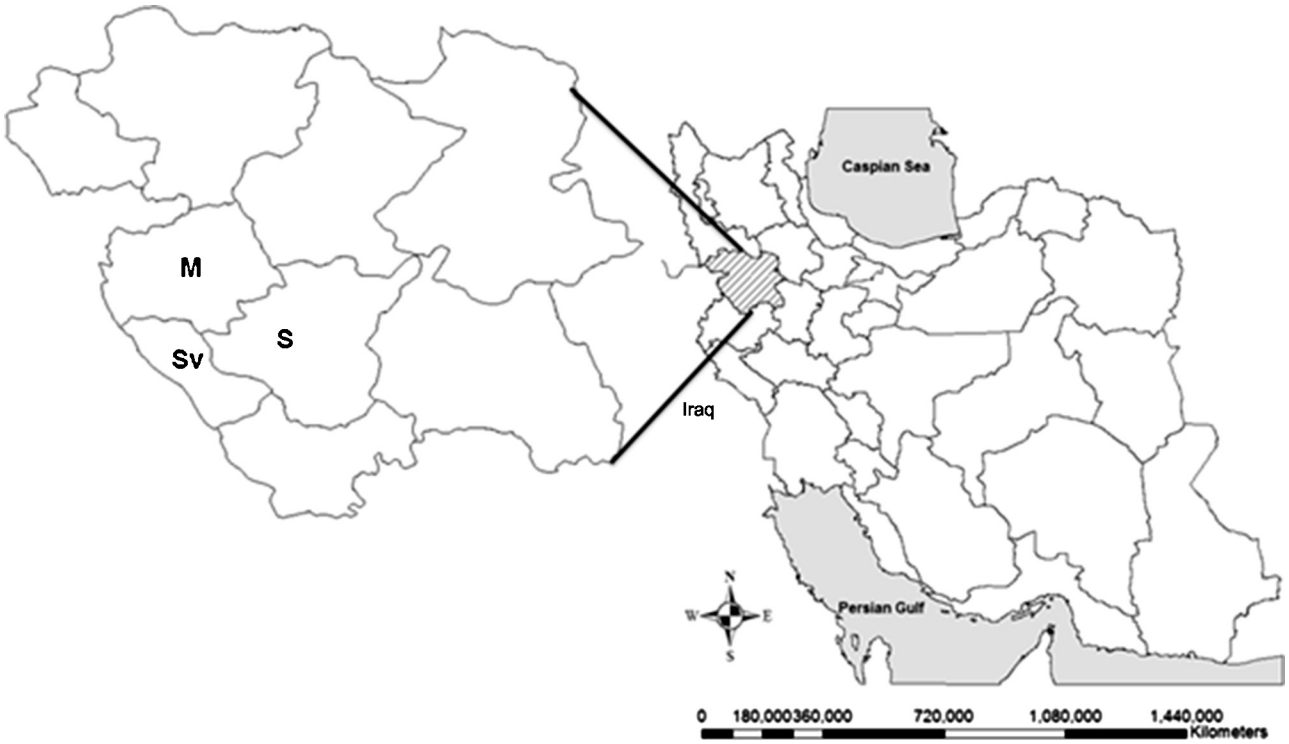

Figure 1. Location of Kurdistan Province on a map of Iran. Sampling was conducted in the counties of Marivan (M), Sarvabad (Sv), and Sanandaj (S) in 2011–2012.

Table 1
Relationship between demographic characteristics and tularemia seropositivity in Kurdistan Province between 2011 and 2012

Variable	Category	No. tested (% seropositive)	*p*-Value	OR (95% CI)
Occupation	Health care workers	50 (12.00)	Reference	
	Hunters	50 (18.00)	0.40	1.61 (0.53–4.92)
	Butchers	50 (16.00)	0.57	1.40 (0.45–4.37)
	Those referred to medical diagnostic laboratories	100 (13.00)	0.86	1.10 (0.39–3.08)
Age, years	18–30	71 (7.04)	Reference	
	31–40	69 (8.70)	0.72	1.26 (0.37–4.33)
	40–50	58 (18.96)	0.05	3.09 (1.01–14.56)
	≥51	52 (26.92)	0.01	4.86 (1.63–14.56)
Gender	Female	44 (15.91)	0.75	0.87 (0.35–2.13)
	Male	206 (14.08)		
Area of residence	Urban	51 (7.84)	0.14	2.25 (0.76–6.69)
	Rural	199 (16.08)		

OR, odds ratio; CI, confidence interval.

Germany) in accordance with the manufacturer's instructions. Based on the optical density (OD) obtained, the results were divided into three categories: positive, negative, and borderline. Positive and borderline samples were tested to investigate the possibility of a cross-reaction with brucellosis by standard tube agglutination test (diagnosis kit produced by the Pasteur Institute of Iran).

2.3. Statistical analysis

The data were analyzed using SPSS software version 16 (SPSS Inc., Chicago, IL, USA), and the Chi-square, Fisher's exact test, and regression logistic tests were used to compare the variables during the analysis. A p-value of <0.05 was considered statistically significant.

3. Results

In this study, 100 samples from referrals to medical diagnostic laboratories, 50 samples from hunters and their families, 50 samples from health care workers, and 50 samples from slaughterhouse workers and butchers were collected from the counties of Sarvabad, Marivan, and Sanandaj.

Eighty-two percent of the participants were men. The average (standard error) age of participants was 40.15 (0.82) years. The median length of time of employment for participants (hunters, health care workers, and butchers) was 10 years. Twenty-six percent of participants found themselves at risk of zoonotic diseases.

Forty-two percent of participants had kept domestic animals. Of this number, 86.45% had kept cattle, 59.62% had kept sheep or goats, and 3.85% had kept dogs or cats. Thirty-three percent of them had hunted wild animals and 58.40% had eaten the meat of wild animals. Partridge (91.89%), rabbit (82.31%), and fox (29.73%) were the most hunted or eaten animals by the participants in this study.

From all 250 sera, 36 samples (14.40%) had anti-tularemia IgG antibodies and 33 samples (13.20%) had borderline tularemia antibodies. Among the positive cases, only one of the samples was simultaneously positive for brucellosis.

Tularemia seropositivity in the study groups in Sanandaj, Sarvabad, and Marivan counties was 8.70%, 16.18%, and 4.35%, respectively; these differences were not statistically significant ($p = 0.19$). The highest seroprevalence was found in hunters (18%) and the lowest in health care workers (12%). Age had a significant positive association with the seropositivity rate of tularemia ($p < 0.001$); for every increase in age by 1 year, the chance of being seropositive increased 1.05 times (odds ratio 1.05, 95% confidence interval 1.02–1.08). The highest seroprevalence of tularemia was in the age group ≥51 years (26.92%). The difference in the seropositive rate between males (14.08%) and females (15.91%), and also between rural (16.08%) and urban (7.84%) residents, was not statistically significant ($p = 0.75$ and $p = 0.14$, respectively) (Table 1).

The seroprevalence of tularemia in individuals exposed to foxes (hunting or eating the meat) (25%) was significantly higher than in others (8.65%) ($p = 0.01$). Length of employment was also a risk factor for the seroprevalence rate of tularemia, and those in employment for more than 10 years had significantly higher levels of seropositivity than those in employment for less than 10 years ($p = 0.02$). Other variables had no significant influence on tularemia seroprevalence among these individuals (Table 2).

4. Discussion

This study is the first carried out on tularemia among different populations in Iran and showed a high seroprevalence in the west of Iran. However, there has only ever been one case of clinical tularemia in Iran (1980).[16] The results of other studies have also shown that tularemia exposure does not usually lead to severe or significant clinical symptoms[18] and the populations in endemic areas have measurable rates of antibodies to tularemia.[3] The comparison of the two subspecies of *F. tularensis* influences the results of our study as well, as the circulating subspecies in our study area should be type B and the clinical symptoms and virulence of type B disease are at a lower level than type A.[7]

A lack of attention by physicians to tularemia in the differential diagnosis of diseases with similar clinical symptoms, and blind treatment with antibiotics in probable patients, could explain the lack of clinical cases of tularemia in this region, despite the high seroprevalence in our study. Other possibilities are that the routes of exposure, the infection dose, and the virulence of the organism in the study area may be more likely to produce an asymptomatic infection in this population.

The rate of tularemia seroprevalence in this study (14.40%) was higher than in other studies, where rates among high-risk populations have been reported at 2% in Germany,[19] 2% in Canada,[20] 0.3–6.3% in Turkey,[21–23] and 9% in the USA.[3] However, in a study carry out in the Republic of Azerbaijan, the seroprevalence rate was 15.5%,[18] which is higher than that found in the present study.

In this study the highest tularemia seroprevalence was observed in hunters (18%). Generally, hunters as a group are considered to be at high risk of tularemia infection. They may become infected with tularemia by hunting or contact with wild animals, and one study in Germany has shown that the seroprevalence of tularemia among hunters (1.7%) is higher than in the general population (0.2%).[19] The seroprevalence of tularemia among hunters was found to be 6.3% in Turkey,[23] which is lower than the observation in our study. There is no similar information

Table 2
Relationship between behavioral characteristics and tularemia seropositivity in Kurdistan Province between 2011 and 2012

Variable	Number having the variable (% positive)	Number not having the variable (% positive)	*p*-Value
Attitude[a]	66 (13.64)	184 (14.67)	0.84
Splashing animal fluids on face/body	135 (15.55)	15 (13.33)	0.99
Exposure to ill or dying animals	160 (15.00)	90 (13.33)	0.72
Hunting/consumption of wild animal meat	148 (13.51)	102 (15.69)	0.63
Rabbit	121 (14.88)	27 (7.41)	0.53
Partridge	136 (14.71)	12 (0.00)	0.37
Fox	44 (25.00)	104 (8.65)	0.01
Hedgehog	19 (26.32)	131 (11.63)	0.14
Birds[b]	30 (23.33)	118 (11.02)	0.13
Squirrel	14 (0.00)	134 (14.92)	0.22
Badger	15 (26.67)	133 (12.03)	0.12
Other[c]	39 (12.82)	107 (14.02)	0.85
Keeping animals	105 (18.09)	145 (11.72)	0.16
Cattle	90 (17.78)	14 (21.43)	0.72
Goats and sheep	62 (12.90)	42 (26.19)	0.09
Dogs and cats	4 (0.00)	100 (19.00)	0.99
Using disinfection tools	25 (12.00)	74 (14.86)	0.99
Disinfection of hands/face	21 (14.29)	78 (14.10)	0.99
Length of employment (10 years or more)[d]	64 (21.87)	77 (7.79)	0.02
Cutting hand/year (5 times or more)	93 (17.20)	47 (8.51)	0.16

[a] See themselves as at high risk for zoonotic diseases.
[b] Emigrant/feral birds.
[c] Includes weasel, mongoose, jackal, wild boar, and other wild animals.
[d] Median length of time was 10 years.

available on the other occupational groups (butchers and health care workers) included in this study.

In our study, there was a significant positive correlation between age and tularemia seroprevalence, which is similar to the findings of another study.[18] This can be explained by the fact that the tularemia antibodies remain in the body for 10–20 years after the initial infection and the probability of exposure to pathogens increases with age.[12,13] In this study, length of employment also had a positive significant correlation with the rate of tularemia seroprevalence, which was found to parallel the influence of age.

Although we had expected that the seroprevalence of tularemia would be higher in males as a result of more frequent contact with animals and more exposure to ticks,[21] there was no difference between males and females in tularemia seroprevalence, which is similar to the findings of another study carried out in the Republic of Azerbaijan.[18] Rural residents also had twice the rate of tularemia seroprevalence as urban residents, but this difference was not statistically significant either.[22] Generally, residence in rural areas is an important risk factor for tularemia, as shown in a study carried out in Turkey.[21] One of the reasons for the lack of difference in tularemia seroprevalence between genders (females and males) and place of residence (urban and rural) may be the lower number of females and urban residents, which subsequently reduced the effect of the statistical analysis for these comparisons.

The ethnic situation in the area of our study must also be taken into account, as eating the meat of hunted animals is more usual there than in other regions of Iran. Hence one of the factors in our study was the type of animal hunted. Although there was no significant correlation between the seroprevalence of tularemia and hunting or eating the meat of wild animals, hunting or eating fox meat was an important risk factor for a seropositive result for tularemia, insomuch as a seropositive result in individuals exposed to foxes was three times higher than in others. Other studies have shown foxes to be important indicators for assessing tularemia in wildlife.[24–26] Although foxes do not suffer from any notable disease after exposure to the causative agent of tularemia (by eating infected animals such as rodents and rabbits, or exposure to their ectoparasites), this bacterium can remain in the bodies of these foxes and their ectoparasites for a long period time.[27] If humans are exposed to these animals and their ectoparasites during this period, tularemia infection is possible in hunters and others who are in contact with foxes.

Although it was mentioned that there would be a probable interaction between brucellosis and tularemia in serological tests,[28] in our study only one out of 36 positive samples for tularemia IgG (2.77%) was positive for brucellosis.

One of the limitations of this study was the lack of a proper group to represent the general population. Although the ELISA test is a test with high sensitivity and specificity for tularemia IgG diagnosis and this test is used for seroepidemiological studies and primary screening of patients, it is preferable to use complementary tests such as microagglutination, Western blot, and indirect immunofluorescence to confirm positive cases;[14,29] however these complementary tests were not available for this study.

It is suggested that the types of tularemia circulating in Iran are evaluated in future studies. Similar studies in other parts of the country and on domestic and wild animals will help to clarify the epidemiology of tularemia in Iran.

According to the findings of this study, it is highly recommended that physicians and health care workers are informed about bacteria circulating in this area. By sensitizing the health system, it is expected that some cases of the clinical disease will be reported in the near future.

Acknowledgements

We appreciate the financial support of the Pasteur Institute of Iran and the Center for Disease Control of the Iranian Ministry of Health and Medical Education (Grant No. 582). We would like to express our gratitude to Mr Karimi, Mr Bahmani, and Mr Faraji from Kurdistan University of Medical Sciences for their support in the sampling.

Conflict of interest: The authors declare that they have no competing financial interests.

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